

**THE SAME CLAIM
BUT HAVE EXAMINERS' HANDWRITING ON IT**

CLAIMS

1. A method for obtaining transgenic plants having an increased capacity to synthesize, to accumulate and to exude organic acids, by integration into their genome of a recombinant heterologous DNA molecule encoding enzymes that synthesize organic acids, involving the following steps:

(a) preparation of a recombinant heterologous DNA molecule encoding one or more genes for enzymes that synthesize organic acids, linked to a promoter sequence functional in plants, and to a transcription termination/polyadenylation sequence functional in plants;

(b) the transformation of plant cells with the recombinant DNA molecule, and

(c) the regeneration of transgenic plants starting from transformed cells, or of seeds from plants obtained from these transformed cells, for one or several generations, wherein the genetic information of these transformed cells, includes the recombinant DNA molecule coding for enzymes that synthesize organic acids.

2. The method according to claim 1, in which the recombinant DNA molecule comprises one or more microbial genes coding for enzymes that synthesize organic acids.

3. The method according to claim 1, wherein the recombinant DNA molecule comprises a gene of plant origin coding for an enzyme that synthesizes organic acids.

4. The method according to claim 1, wherein the recombinant DNA molecule comprises a gene of animal origin coding for an enzyme that synthesizes organic acids.

5. The method according to claim 2, wherein the recombinant DNA molecule comprises one or more bacterial genes that code for an enzyme that synthesizes organic acids.

6. The method according to claim 1, wherein the recombinant molecule comprises a gene that codes for the enzyme Citrate Synthase.

7. The method according to claim 1, wherein the recombinant molecule comprises a gene that codes for the enzyme Malate dehydrogenase.

8. The method according to claim 1, wherein the enzyme that synthesizes organic acids is located in the cytoplasm.

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112-184(?)

Heininger

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9. The method according to claim 1, wherein the enzyme that synthesizes organic

¹⁰³
+Chou acids is located in chloroplasts.

10. The method according to claim 1, wherein the enzyme that synthesizes organic

¹⁰³
+Chou acids is located in the mitochondria.

11. The method according to claim 5, wherein the recombinant molecule comprises a

¹⁰²
delta gene of *Pseudomonas aeruginosa* that codes for Citrate Synthase.

12. The method according to claim 1, wherein the transcription termination sequence

¹⁰²
delta is the transcription termination sequence of the Nopaline Synthetase gene.

13. The method according to claim 2, wherein the transcription termination sequence

¹⁰²
delta is the transcription termination sequence of the Nopaline Synthetase gene.

14. The method according to claim 3, wherein the transcription termination sequence

¹⁰³
+Chou is the transcription termination sequence of the Nopaline Synthetase gene.

15. The method according to claim 4, wherein the transcription termination sequence

¹⁰³
+Chou is the transcription termination sequence of the Nopaline Synthetase gene.

16. The method according to claim 5, wherein the transcription termination sequence

¹⁰²
delta is the transcription termination sequence of the Nopaline Synthetase gene.

17. The method according to claim 6, wherein the transcription termination sequence

¹⁰²
delta is the transcription termination sequence of the Nopaline Synthetase gene.

18. The method according to claim 7, wherein the transcription termination sequence

¹⁰³
+Chou is the transcription termination sequence of the Nopaline Synthetase gene.

19. The method according to claim 8, wherein the transcription termination sequence

¹⁰²
delta is the transcription termination sequence of the Nopaline Synthetase gene.

20. The method according to claim 9, wherein the transcription termination sequence

¹⁰³
+Chou is the transcription termination sequence of the Nopaline Synthetase gene.

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tChou 21. The method according to claim 10, wherein the transcription termination sequence is the transcription termination sequence of the Nopaline Synthetase gene.

102
dele 22. The method according to claim 1, wherein the promoter is a constitutive promoter.

102
dele 23. The method according to claim 2, wherein the promoter is a constitutive promoter.

103
tChou 24. The method according to claim 3, wherein the promoter is a constitutive promoter.

103
tChou 25. The method according to claim 4, wherein the promoter is a constitutive promoter.

102
dele 26. The method according to claim 5, wherein the promoter is a constitutive promoter.

102
dele 27. The method according to claim 6, wherein the promoter is a constitutive promoter.

103
tChou 28. The method according to claim 7, wherein the promoter is a constitutive promoter.

102
dele 29. The method according to claim 8, wherein the promoter is a constitutive promoter.

103
tChou 30. The method according to claim 9, wherein the promoter is a constitutive promoter.

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tChou 31. The method according to claim 10, wherein the promoter is a constitutive promoter.

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de la
+ Gray
32. The method according to claim 1, wherein the promoter is a root-specific promoter.
33. The method according to claim 2, wherein the promoter is a root-specific promoter.
- + John 34. The method according to claim 3, wherein the promoter is a root-specific promoter.
- + Silvana 35. The method according to claim 4, wherein the promoter is a root-specific promoter.
36. The method according to claim 5, wherein the promoter is a root-specific promoter.
37. The method according to claim 6, wherein the promoter is a root-specific promoter.
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+ John 38. The method according to claim 7, wherein the promoter is a root-specific promoter.
39. The method according to claim 8, wherein the promoter is a root-specific promoter.
- + John 40. The method according to claim 9, wherein the promoter is a root-specific promoter.
- + John 41. The method according to claim 10, wherein the promoter is a root-specific promoter.
- 103
Muehler 42. The method according to claim 1, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

43. The method according to claim 2, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

John 44. The method according to claim 3, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

+ Selman 45. The method according to claim 4, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

46. The method according to claim 5, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

47. The method according to claim 6, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

103 *John* 48. The method according to claim 7, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

49. The method according to claim 8, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

Alton 50. The method according to claim 9, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

Alton 51. The method according to claim 10, wherein the promoter is a promoter inducible by stress caused by low Phosphate availability.

103 52. The method according to claim 1, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

Crüner 53. The method according to claim 2, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

103 + John
 54. The method according to claim 3, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

+ So! 55. The method according to claim 4, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

56. The method according to claim 5, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

57. The method according to claim 6, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

103 John
~~103~~ 58. The method according to claim 7, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

59. The method according to claim 8, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

John 60. The method according to claim 9, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

61. The method according to claim 10, wherein the promoter is a promoter inducible by stress caused by low Iron availability.

102 Dela
 62. The method according to claim 1, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

102 Dela
 63. The method according to claim 2, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

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 64. The method according to claim 3, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

103 + Saluema
65. The method according to claim 4, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

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Dela
66. The method according to claim 5, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

102
Dela
67. The method according to claim 6, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

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68. The method according to claim 7, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

102
Dela
69. The method according to claim 8, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

103
+ Chau
70. The method according to claim 9, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

103
Dela
71. The method according to claim 10, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

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Lundquist
+ Chau
72. The method according to claim 9, wherein the recombinant molecule comprises a signal peptide sequence to direct a heterologous enzyme that synthesizes organic acids to the chloroplast or the mitochondria of the transgenic cells.

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Lundquist
+ Chau
73. The method according to claim 10, wherein the recombinant molecule comprises a signal peptide sequence to direct a heterologous enzyme that synthesizes organic acids to the chloroplast or the mitochondria of the transgenic cells.

102!
Dela
74. A recombinant heterologous DNA molecule comprising one or more genes that code for enzymes that synthesize organic acids, functionally linked to a promoter sequence functional in plants, and to a transcription termination/polyadenylation sequence functional in plants.

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della 75. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids, is from one or more microbial genes.

103 76. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids is from a gene of plant origin.

John
103 + Sherman 77. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids is from a gene of animal origin.

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della 78. The recombinant DNA molecule according to claim 74, wherein the coding sequence for the enzyme that synthesizes organic acids, is from one or more bacterial genes.

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della 79. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is a gene that codes for the enzyme citrate synthase.

102
della 80. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is a gene of *Pseudomonas aeruginosa* that codes for the enzyme citrate synthase.

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Uchi 81. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is a gene that codes for the enzyme Malate deshydrogenase. Sp

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della 82. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is an enzyme that is located in the cytoplasm.

103
+ Chou 83. The recombinant DNA molecule according to claim 74, wherein the gene that codes for the enzyme that synthesizes organic acids is an enzyme that is located in the chloroplast.

102 84. The recombinant DNA molecule according to claim 74, wherein the promoter is a constitutive promoter.

103 + Croy 85. The recombinant DNA molecule according to claim 74, wherein the promoter is a root-specific promoter.

103 + Muechd 86. The recombinant DNA molecule according to claim 74, wherein the promoter is a promoter whose expression is inducible by stress caused by low phosphate availability.

103 + Croy 87. The recombinant DNA molecule according to claim 74, wherein the promoter is a promoter whose expression is inducible by stress caused by low iron availability.

103 + Croy 88. The recombinant DNA molecule according to claim 74, wherein the promoter is the 35S promoter of the cauliflower mosaic virus.

103 + Croy 89. The recombinant DNA molecule according to claim 74 comprising a sequence that includes a transit peptide sequence for chloroplast or mitochondrial protein targeting in plants.

103 + Croy 90. The recombinant DNA molecule according to claim 74, comprising a transcription termination/polyadenylation sequence that is the transcription termination/polyadenylation sequence of the Nopaline Synthetase gene.

102 91. The recombinant DNA molecule according to claim 74, as defined in figure 1.

102 92. The vector comprising the recombinant DNA molecule according to claim 74.

102 93. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 74.

102 94. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 75.

103
 95. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 76.
 + John

103
 96. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 77.
 + Silberman

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 97. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 78.
 Dele

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 98. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 79.
 Dele

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 99. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 80.
 Dele

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 100. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 81.
 John

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 101. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 82.
 Dele

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 102. Transgenic plants with increased capacity to synthesize, to accumulate and to exude organic acids by integration into their genome of a recombinant heterologous DNA molecule as defined in claim 83.
 + Chad
 Chad

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Dela 103
111. Transgenic plants according to claim 93, tolerant to toxic concentrations of Aluminum.

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Dela 104
112. Transgenic plants according to claim 93, having increased capacity to solubilize or accumulate phosphate.

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Bela 105
113. Transgenic plants according to claim 93, having increased capacity to solubilize or accumulate iron.

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Bela 106
114. Transgenic plants according to claim 93, requiring less fertilizer for their growth.

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115. Transgenic plants according to claim 93, that develop better or have higher productivity in acid soils.

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116. The transgenic plants according to claim 93, wherein the plant is a monocotyledonous plant.

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117. Transgenic plants according to claim 93, wherein the plant is a dicotyledonous plant.

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Ob. 110
118. Transgenic plants according to claim 115, wherein the plant belongs to anyone of the families: *Pyc Grass* Poaceae or Lileaceae.

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Ob. 111
119. Transgenic plants according to claim 117, wherein the plant belongs to anyone of the families: Leguminoseae, Solanaceae, Caricaceae or Cucurbitaceae.

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Ob. 112
120. Transgenic plants according to claim 116, wherein the plant belongs to any of the species: Triticum spp, Oryza sativa, Zea mays, Sorghum bicolor, Avena sativa or Saccharum officianarum.

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Ob. 113
121. Transgenic plants according to claim 117, wherein the plant belongs to any of the species: Solanum tuberosum, Lycopersicum esculentum or Glycine max.

112 and compared to what?

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122. Transgenic plants according to claim 117, wherein the plant is of the Nicotiana genus.

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123. Transgenic plants according to claim 122, wherein the plant is of the Nicotiana tabacum species.

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124. Transgenic plants according to claim 117, wherein the plant is of the Carica genus.

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Papaya

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125. Transgenic plants according to claim 124, wherein the plant is of the Carica papaya species.

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126. Use of the transgenic plants according to claim 81 in acid soils.

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127. Use of the transgenic plants according to claim 81 in soils containing phosphates in forms not available for the plant nutrition.

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128. Use of the transgenic plants according to claim 81, for practice or cultivation systems that use less fertilizer.

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129. The transgenic seeds or any vegetative reproductive structure attainable from a transgenic plant as defined in the claim 81.

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130. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 74.

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131. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 75.

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124

132. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 76.

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125

133. A transformed cell or protoplast transformed with the recombinant DNA molecule as defined in claim 77.

John

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Silvema

¹³⁴
134. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 78.

¹³⁵
135. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 79.

¹³⁶
136. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 80.

¹³⁷
137. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 81.

¹³⁸
138. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 82.

¹³⁹
139. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 83.

¹⁴⁰
140. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 81.

¹⁴¹
141. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 85.

¹⁴²
142. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 86.

¹⁴³
143. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 87.

¹⁴⁴
144. A transformed cell or protoplast transformed with the recombinant DNA molecule
as defined in claim 88.

identical

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as defined in claim 90.